

From the Dermatological Clinic of the University of Zurich

Director: Prof. Dr. G. Miescher

**A Study by Means of Paper Electrophoresis of the Changes
in the Protein Components of Guinea Pig Serum during
Sensitization to Horse Serum and after Anaphylactic Shock**

INAUGURAL-DISSERTATION

FOR THE

DEGREE OF DOCTOR OF MEDICINE

FROM THE

MEDICAL FACULTY OF

THE

UNIVERSITY OF ZURICH

PRESENTED BY

ROY GEDULDIG

OF NEW YORK

ACCEPTED ON THE PROPOSAL OF
PROF. DR. G. MIESCHER

ZURICH 1954
BRUNNER & BODMER



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INTRODUCTION

In a previous study reported by H. GEDULDIG (1), an attempt was made to determine whether any changes in the serum proteins of guinea pigs could be detected by means of paper electrophoresis during sensitization and production of experimental contact eczema. As reported, the interpretation of the results was not clear. Due to the large experimental error in the method, it could not be determined whether the small changes which were observed were significant or not. However in contact eczema, no free antibodies have ever been conclusively demonstrated, and therefore the likelihood of finding significant changes in the serum proteins was not to be awaited.

On the other hand, the existence of antibodies in anaphylaxis is readily demonstrated. The ease with which passive transfer (2) and the SCHULTZ-DALE phenomenon (3, 4) can be carried out are accepted signs of the presence of antibodies.

Therefore in anaphylaxis where antibodies are known to exist, one might theoretically expect to find some changes in the serum proteins. It is the object of this paper, therefore, to determine whether any changes in the protein fractions of guinea pig serum during anaphylaxis can be detected, and to evaluate the method of paper electrophoresis for experimental studies.

EXPERIMENTAL

Young male guinea pigs weighing from 300 to 400 grams were selected. The animals were divided into three groups.

A first group consisting of 18 animals was inoculated intraperitoneally with 1, 2 and 3 ccm of horse serum on three successive days.

On the thirteenth and seventeenth day respectively after the first injection the shock was produced either by intraperitoneal or intracardial reinjection. With intraperitoneal reinjection the animals showed small or no signs of shock, while

with intracardial reinjection the signs of shock were decidedly pronounced and most of the animals died.

A second group consisting of 10 animals was inoculated intracardially with a single dose of 0.5 ccm horse serum. The shock dose (0.5 - 0.75 ccm) was given intracardially too on the twelfth day. Eight showed signs of shock and two died from it. Eight days later, five surviving animals were given 1.0 ccm serum intracardially. All five showed signs of shock and three died. These all showed the typical autopsy finding of an anaphylactic shock.

During the sensitization period of all animals, 1 ccm of cardiac blood was taken daily from three animals at a time. Different animals were taken every day to allow them time to recuperate from the repeated cardiac punctures.

Before the animals were given a shocking dosage intracardially, blood was always withdrawn for investigation so that a comparison could be made of the serum before and after the shock. While the needle remained in the heart, syringes were switched and then horse serum injected. After the shock, blood was again taken. If the shock proved fatal, the autopsy was performed immediately and blood was taken from the heart, which was usually still beating. If the animal survived, blood was taken about one-half hour after the shocking dosage was given.

The serum thus obtained was used for the electrophoretic studies. This was done by means of the paper electrophoresis method of GRASSMANN (5). All sera were run in duplicate.

As controls, curves were also run on normal animals.

An attempt was made to determine the serum proteins by means of the paper electrophoresis, of the horse serum used. (The possibility was considered that the injection of up to 1 ccm. of the horse serum directly into the bloodstream of the guinea pig might cause a change in the electrophoretic results simply due to the physical properties of the added serum). However no separation of the globulin fractions of the horse serum could be accomplished by the paper electrophoresis. Nevertheless, an examination of the electrophoretic curves does seem to indicate a high globulin content of the horse serum.

RESULTS

Since the range of values for normal animals is very great and no definite trend could be observed during the course of the experiment, an attempt was made to combine the results of the various parts of the experiment so that they could be compared statistically. These results are summarized in Table I.

Standard deviation was determined by the formula:

$$\sigma = \pm \sqrt{\frac{\sum (x - \bar{x})^2}{n}}, \text{ where}$$

x = individual result

$\bar{x}$ = the mean

n = number of individual results

Results included in the sensitization period extend from one day after the first dose of horse serum was given to the time when the first anaphylactic dose was given.

That column entitled "Sensitized animals before shocking dose was given" included curves from all animals that had already survived at least one shocking dose (with or without reaction) and was from the blood obtained immediately before the shocking dose of horse serum was injected intracardially.

The last column entitled "Animals with definite signs of shock" is a heterogeneous group. It includes all animals regardless of the method of sensitization, whether the shock dosage was given intraperitoneally or intracardially, or whether the animals survived or not, as long as the classical signs of anaphylactic shock were displayed. (For a further breakdown of this column, see the Discussion).

The classical description of ANDERSON (6) was used as a criterion to determine whether anaphylactic shock occurred. Usually after a latent period of about three minutes, the animal begins to show signs of discomfort. The animal rubs his nose with his paws and may sneeze. He then has tonic-clonic cramps, or may actually jump wildly about the box. In another minute, he urinates, falls on his side, shows an acute air hunger, and dies shortly thereafter. In the non-fatal cases, the cramps and air hunger are usually, but not always, weaker.

Summary of results Table 1

	Albumin	α	Globulins β	γ
normal	47, 8 $\pm$ 3, 5	27, 1 $\pm$ 1, 2	8, 5 $\pm$ 1, 4	16, 6 $\pm$ 3, 5
Sensitized intraperitoneally 18 animals	49, 3 $\pm$ 3, 5	26, 0 $\pm$ 2, 6	8, 5 $\pm$ 1, 2	16, 6 $\pm$ 3, 3
Sensitized intracardially 10 animals	42, 9 $\pm$ 2, 8	28, 6 $\pm$ 2, 7	9, 3 $\pm$ 1, 4	19, 2 $\pm$ 4, 4
Sensitized animals before shocking dose	44, 4 $\pm$ 2, 4	28, 3 $\pm$ 3, 2	9, 1 $\pm$ 2, 0	18, 2 $\pm$ 3, 4
Shock dose intraperitoneally no reaction	45, 9 $\pm$ 2, 7	27, 9 $\pm$ 2, 5	9, 2 $\pm$ 2, 2	17, 0 $\pm$ 2, 4
Shock dose intraperitoneally no reaction	45, 8 $\pm$ 5, 0	26, 9 $\pm$ 2, 6	10, 0 $\pm$ 2, 2	17, 3 $\pm$ 2, 9
Animals with definite signs of shock	43, 7 $\pm$ 4, 3	29, 2 $\pm$ 3, 7	9, 3 $\pm$ 2, 0	17, 8 $\pm$ 3, 7

On autopsy the typical pathological findings (7) could be seen. The lungs were maximally expanded and congested. The heart could be seen beating long after respiration had ceased.

Many of the curves of the group entitled "Shock dose intracardial, no reaction" were from animals that had died of hemorrhages. The differentiation between shock due to bleeding and one due to anaphylaxis is not always absolutely sharp. In general, the former begins earlier, often while the horse serum is still being injected. The animal usually runs around wildly, falls on his side, and appears to have an air hunger. On autopsy the lungs are contracted and a hemopericardium or hemothorax are found.

However in some cases, the shock was not characteristic of either type. When the animal died, autopsy often revealed maximally expanded lungs with a profuse hemorrhage in addition. Such cases were considered as a definite sign of anaphylactic shock. HOIGNE (8) reported an immediate sharp rise in the antithrombin with a simultaneous drop of thrombocytes upon injecting the anaphylactic dose. Since the animals had approximately five heart punctures before receiving the shocking dose of horse serum, the damaged heart muscle plus this disturbance of coagulation could very well explain the high percentage of hemorrhages that resulted. Probably a few that were diagnosed as not having had an anaphylactic shock, with a heart tamponade and contracted lungs, died too rapidly for the anaphylactic symptoms to take effect.

DISCUSSION

From Table I, it can be seen that no change is apparent during the sensitization period if the guinea pig has been inoculated intraperitoneally.

However, in the animals sensitized intracardially, there appears to be a significant drop in the albumin with a rise in the globulin values, especially in the γ globulin. To determine whether this change was of statistical significance, it was decided to find "t", which is an arbitrary value used to determine whether a significant difference exists between two groups of results.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sum (x_1 - \bar{x}_1)^2}{n_1} + \frac{\sum (x_2 - \bar{x}_2)^2}{n_2}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \quad \text{where}$$

x_1 = individual values in group of normal animals

- $\bar{x}_1$ = mean value for the normal animals
 n = number of individual values of the normal group
 x_2 = individual values in the group to be compared
 $\bar{x}_2$ = mean value of group to be compared
 n_2 = number of individual values in this group

From the formula, it was found that for the albumin the t value was 4.9. This indicates that it is very probably of statistical significance. The t value for the γ globulin was 2.5, which gives it little statistical significance.

If these values for the various protein fractions are of significance, one should expect to find the same, or even a more pronounced dysproteinemia, after a shocking dose was given. As has been stated, the column entitled, "Sensitized animals before shocking dose was given" is a heterogenous group of all the animals that had survived at least one anaphylactic shock dose. A breakdown of these figures into animals that had been sensitized intracardially and those sensitized intraperitoneally gives the following results:

	Alb.	α	β	γ
intracardially sensitized	41.5%	29.4%	8.7%	20.4%
intraperitoneally sensitized	45.3%	28.0%	9.2%	17.5%

It thus appears that although all the animals of this group had increased globulins, the animals that had been previously sensitized intracardially show an even greater dysproteinemia. The values for the latter show a further increase in the globulins as compared to the values obtained during the sensitization, which seems to bear out the fact that a change has taken place.

To follow the breakdown to its logical end, the protein values determined by electrophoresis of the animals who showed "signs of shock" were divided into those:

- 1) Sensitization and shock intraperitoneally - lived
 Albumin 51.3% α 29.0% β 8.5% γ 11.2 rel. %
- 2) Sensitization and shock intraperitoneally - died
 Albumin 45.6% α 26.2% β 10.1% γ 18.1 rel. %
- 3) Sensitization intraperitoneally, shock intracardially - lived
 Albumin 43.3% α 29.5% β 9.9% γ 17.3 rel. %
- 4) Sensitization intraperitoneally, shock intracardially - died
 Albumin 45.4% α 28.9% β 9.4% γ 16.2 rel. %

5) Sensitization and shock intracardially - lived

Albumin 40.2% α 30.4% β 9.7% γ 19.7 rel.%

6) Sensitization and shock intracardially - died

Albumin 42.8% α 28.6% β 9.1% γ 19.5 rel.%

It can be seen that here too the results of the group sensitized intracardially correspond closely to those obtained during the sensitization. All this indicates that the results obtained with this group of animals was not merely accidental, but that the probability is large that a change in the serum proteins takes place when guinea pigs are sensitized to horse serum intracardially.

Why these changes are observed with intracardial injection and not with intraperitoneal is extremely puzzling. Assuming that the guinea pig has 25 ccm. of blood the 1 ccm of horse serum added could not explain the large difference observed.

An interesting observation is that the animals sensitized intraperitoneally show a decided increase in the globulin fraction when examined in the two columns, "Sensitized animals before shocking dose was given" and "Animals with definite signs of shock". However all animals in the former column had at least one attempted anaphylactic shock, as well as many in the latter column. Since many of these previous injections attempting to cause anaphylactic shock were intracardial, many of these animals can be considered to have received an intracardial sensitization as well.

The interpretation of the protein changes is difficult. Since all the results are given in relative percentages, if the globulins increase, the albumin must naturally decrease. It is also seen that the increase in globulin is not due to an increase in a single fraction, but that the increase is divided between the α and γ globulins. If the entire increase of the globulins is attributed to antibody formation, then the antibodies apparently do not migrate solely with the γ globulins as is commonly accepted. However LOVELESS and CANN (9) and CAMPBELL et al (10), working with electrophoresis convection, indicate that antibody activity may be found in the α and β globulin components as well. On the other hand, DOERR (11) states that the changes in the electrophoretic pattern are due to unspecific protein changes and the antibody formation does not necessarily correspond to the changes in the serum proteins.

A still greater difficulty in interpreting the results stems from the method used. Recently RIVA and MARTINI (12) have presented evidence to show that the results obtained with paper electrophoresis are not as dependable as those obtained by use of the TISELIUS method. This has been borne out in the present experiment. All

sera were run in duplicate and it was considered a good check if the absolute difference was not greater than 2 % for any value. Naturally with such a large error in two identical samples, it is difficult to compare different sera, thus explaining the absolute necessity to do a statistical study.

Not only the method leads to considerable error, but the very fact that the guinea pig was used as the experimental animal increases the source of error. Whereas with human serum, the separation by means of paper electrophoresis is sharp, with guinea pig serum a distinct separation, especially of the β & γ globulins is practically impossible to obtain. This means that the Gaussian curves drawn in to interpret the various fractions are very difficult to construct and a large error due to subjective interpretation is introduced. Also since it is not certain with which component the antibody wanders, a good separation is particularly desirable

In addition, the normal values of guinea pig serum vary much more than those of normal human serum. This again necessitates a statistical study in order to determine whether a change has taken place or not. Table II gives a survey of the literature of various values reported for normal guinea pig blood. Not only do the averages reported by the various authors show marked differences from each other, but the range of values reported by the individual authors is also very large. SVENSSON (13) reported albumin values of 46.8-64.4 relative percent, MOORE (14) from 42-66 relative percent, and H. GEDULDIG (1) from 41.6-60.0 relative percent. The author's own albumin results range from 39.1-55.4 relative percent. The author's values for normal animals and those reported by H. GEDULDIG show a considerable discrepancy. This must be put down to the fact that the latter work was done in the summer while the other was done during the winter. During the summer, the main constituent of the diet is fresh grass, whereas the chief constituent in the winter is stored white beets.

As can be seen, the albumin values reported in this paper for normal guinea pig serum are lower than any of those reported in the literature. Whether this indicates a general tendency towards high globulin values in all the sera investigated, and thus tends to refute the findings reported here, cannot be easily discounted. However the fact that the animals sensitized intracardially show a statistically significant change in the protein components from the normals done by the same method at the same time, indicates that some increase in the serum globulins occurs during sensitization to anaphylactic shock. That this change can be seen after the animals have received an anaphylactic shock bears out this conclusion.

A more exact evaluation of the changes in the serum proteins during anaphylactic sensitization awaits a more reliable method and an experimental animal with a smaller range of serum protein values in the normal animal.

A comparison of the mean values expressed in relative percent of electrophoretic studies on serum and plasma of normal guinea pigs

Table 2

Author	Method	No. of animals	Globulins				Fibrinogen
			Albumin	α	β	γ	
SVENSSON (33)	TISELIUS	--	55,8	14,5	8,2	21,5	--
MOORE (27)	TISELIUS	15	54	α_1 11 α_2 21	32	6	8
DEUTSCH & GOODLOE (34)	TISELIUS	3	54,6	α_1 4,0 α_2 3,7 α_3 15,2	22,9	8,8	5,6
Own results	Paper electrophoresis	10 representing 60 curves	50,6 $\pm$ 4,4	26,6 $\pm$ 4,0	8,4 $\pm$ 1,8	14,4 $\pm$ 3,4	--

SUMMARY

1. An attempt to determine whether changes in the serum protein fractions of guinea pigs occurred in sensitization to anaphylactic shock is reported. The study was made with the use of paper electrophoresis.

2. A high probability exists that the globulins increase if the animals are sensitized intracardially, but no change could be ascertained with intraperitoneal sensitization. The increase of globulins occurs in the α and β fractions.

3. A number of sources of error are discussed, which detract from the reliability of the findings.

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CURRICULUM VITAE

I was born in Brooklyn, New York, U.S.A., on July 13, 1926. After attending a number of New York primary and secondary schools, I entered the Polytechnic Institute of Brooklyn in 1942. In 1944 my education was interrupted and I spent the next two years in the United States Navy as an electronic technician's mate. In 1946 I returned to the Polytechnic Institute of Brooklyn where I graduated in June 1948 with the degree of Bachelor of Science in Chemistry.

In the fall of 1948, I entered the third pre-clinical semester of the Medical Faculty of the University of Zurich. All the following semesters were taken at this institute, where in the fall of 1953, I passed the "medizinische Staatsexamen" for foreigners.

